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TI Separation and Analysis of Plasmid Denatured Forms Using **Hydrophobic** Interaction Chromatography
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AB This work explores the possibility of using a **hydrophobic** interaction chromatog. (HIC) support to sep. supercoiled plasmids from denatured forms, by taking advantage of their different surface **hydrophobicity**. The **hydrophobic** gel used in this work was prepd. by covalent immobilization of 1,4-butanediol diglycidyl ether on Sepharose CL-6B (Pharmacia). The **hydrophobic** interaction between this support and lipases was previously reported. Expts. were carried out in a 16 x 150-mm column packed with this gel and equilibrated with 10 mM Tris, pH 8, with 1.5 M (NH4)2SO4 at a flow rate of 60 mL/h. The absorbance was monitored at 254 nm. The plasmid used in the expts. was produced by fermn. of E. coli DH5.alpha. competent cells transformed with the 8.5-kb pCF1-CFTR plasmid (Genzyme Corp.). Growth was carried out overnight in LB medium (30,ug/mL kanamycin), in 100-mL shake-flasks at 37.degree. and 250 rpm. This work shows that HIC can be used for the sepn. of plasmid variants. The technique can play an important role in the preparative purifn. of super-coiled plasmids for gene therapy and DNA vaccination. In fact, the HIC support studied was capable of removing denatured plasmid variants that are usually produced with the widespread method of alk. lysis of plasmid isolation. This is very difficult to achieve using other chromatog. processes. Another important application could be in the monitoring and quality control of purified plasmids. Ongoing work indicates also an ability of the HIC support to sep . **RNA** and genomic **DNA** from **plasmids**. (c) 1999 Academic Press.
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